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TITLE
PROCESS FOR THE FRACTIONATION OF CEREAL BRANS

DESCRIPTION

INTRODUCTION

Cereal Bran is defined as the seed coat of the grains of wheat, barley, rye, triticale, oat or rice. Anatomically, bran comprises the outer layers of the seed, known as the pericarp-testa and an inner layer known as the aleurone layer, which is often classified as the innermost layer of the endosperm. However, bran as defined in this case is the remaining material after the conventional milling, e.g. roller-milling or short roller-milling, of grain and contains both pericarp-testa and aleurone layer components, along with the cereal germ and residual parts of the endosperm. This is the normal understanding of the term "bran" by those working on cereal grain processing.

Within this definition, bran therefore contains all of the pericarp- testa component, the aleurone layer, the germ components including germ proteins and oils, along with a residual amount of endosperm starch, gluten and pentosans.

The invention relates to methods, procedures and an industrial process for the wet-fractionation of bran into two protein rich fractions, one of which contains the germ oils and related components, a fibre fraction which also retains most of the aleurone proteins and a sugar syrup fraction.

The invention is centred around the wet-milling of bran in the presence of enzymes: an alpha amylase, amyloglucosidases, (polysaccharidases ??) and a phytase, under appropriate conditions of temperature, i.e. from 50 to 90, more preferably from 50 to 75, and pH from 4 to 7.5. This is followed by the separation of the above listed components from aqueous suspension using mainly centrifugal separation methods.

Presently there exists no commercial method for wet-fractionating cereal bran to produce the specific products mentioned above.

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PRIOR ART

US patent 4,361,651 describes a process for making fermentable sugars and high protein products from grain, mainly maize. In this method, grain is steeped for 10-30 hours, prior to milling and separation of the germ component, saccharification of carbohydrates (mainly starch), and separation of fibre. The yield of starch is maximised for fermentation to alcohol. Within the described process there is no specific fractionation of the bran component, separation of protein types or consideration of the germ component.

US patent 5,312,636 informs on a process for fractionating crop into industrial raw material. This is focused on oat grain and incorporates bran fractionation procedures that involve the extraction of more hydrophobic components such as lipids in polar organic solvents prior to the alkaline extraction of residual bran to produce beta-glutan, protein and degummed fibres. The use of the organic solvent is a key step in the process and hydrolysing enzymes are not utilised during the fractionation procedure.

US patent 4,746,073 describes a method and apparatus for the physical separation and recovery of an enriched fraction of aleurone cell particles from wheat bran. This is achieved by hammer-milling the bran and then subjecting the resultant particles to a physical separation regime. No wet-processing is employed during the fractionation procedure described therein.

Two related US patents (4,171,383 and 4,171,384) inform on dry and wet milling procedures for refining whole wheat grain. 4,171,383 focuses on wet-milling of the whole kernel. The bran produced is mixed with a separated (mainly) endosperm protein fraction to produce animal feed. 4,171,383 describes dry milling of the whole kernel to produce an endosperm fraction, a germ fraction and a bran fraction. The endosperm fraction is then subjected to wet-milling and separation of starch-rich and protein-rich fractions. The protein rich fraction is added to the bran to produce an animal feed. There is no description of a specific wet fractionation of the bran itself within either patent.

US Patent 3,879,373 describes a process in which pentosans are isolated from wheat bran after alkaline-ethanol extraction of oils, fats, pectins and lignin or chlorine mediated

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delignification. This approach creates several problems, most notably the extreme chemical conditions employed and subsequent treatment of effluent. Furthermore, the use of chlorine in preparing food and feed grade materials is questionable.

US patent 5,308,618 teaches of a process to prepare dietary fibre as an extract from wheat bran. The bran is extracted at high temperatures and pressures in water (180 - 200 C), producing a glucose rich dietary fibre component in the water phase. The process specifically targets the production of dietary fibre and is not really / strictly a fractionation procedure in that other products are largely ignored.

It is clear that none of the abovementioned inventions have arrived at a process of bran cereal fractionation, which is both chemical-free and yields different food-grade fractions.

The main objectives of this invention were to:

1. Arrive at an efficient and cost effective wet process to separate valuable fractions of distinct chemico-physical properties from cereal brans.
2. Combine the use of biological treatment with cell-free enzymes with wet milling to minimise both hydrolysis time and degree of fibre contamination in the non-fibrous fractions.
3. Ensure that in the fractionation process protein fractions of distinct physical properties and therefore functionalities were obtained.
4. The process is carried out in such a way so that use of chemicals extraction procedures are avoided and therefore food grade fractions can be produced.
5. Minimise changes in the nutritive value, structure and functionalities originally observed in the main components, and in particular maintain the antioxidating capacity present in the oil so as to extend the shelf life of the protein-oil fraction.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

It is widely known and accepted that when cereals are milled with the purpose of producing flour the most nutritious part of grain is diverted into the by-product, i.e. cereal bran. Despite the fact that cereal brans are rich in proteins, oils, vitamins and minerals its use in

the food industry and high value feed industry is rather limited. This is primarily because bran, such as wheat bran, contains insoluble fibre.

The inventors have developed an industrial process, which makes possible the separation of fractions of different nature from various cereal brans, produce high value protein and sugar fractions and extract virtually all insoluble fibre as a separate fraction. The resulting low-fibre protein and sugar fractions as well as the insoluble fibre fraction have much broader market applications and greater value than the original bran.

EXAMPLE 1

Wheat bran produced from short milling (SMB) and conventional milling (CMB) processes were used in this trial. Bran sample of 25 kg was transferred to a mixing tank and sequentially hydrolysed at temperatures varying from 70 °C at the first stage with α-amylase to 60 °C in the second stage with amyloglucosidase for a total hydrolysis time of 4 h. During this period the reaction mixture was intermittently wet milled to increase in surface area and dispersion of soluble components. The pH of the reaction mixture was set at neutral initially and then decreased down to 4.5 with acetic acid in the second stage. In addition of maximising the enzymic activity the acidic pH allowed partial solubilization of the phytates present in the bran.

At the end of the enzymic hydrolysis - wet milling step the enzymes contained in the reaction mixture were inactivated by wet heating through a heat exchange and quickly cooled down to room temperature.

The hydrolysed bran solution was then put through a two phase decanter to separate the insoluble (fibre and aleurone fractions) from the soluble fraction.

The soluble fraction was put through a separator so that the heavy phase containing mostly the germ components could be separated from the light phase containing mostly components from the remaining endosperm found in the bran. The light fraction, which was heavily contaminated with sugars, was put through an ultrafilter having a 50 kDa filter in order to separate low molecular weight sugars and a protein fraction with less sugar contamination from each other.

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All soluble protein fractions, i.e. heavy and light phases, were blended together and finally processed through spray drying. The sugar fraction was concentrated by vacuum evaporation at mild temperature (40 to 60 °C) until a 75% sugar concentration was achieved. The fibre fraction was dried in a conventional laboratory oven, but in an industrial process this can be carried out by a number of different drier, i.e. tumble drier, ring drier, fine grinder, etc.

Average chemical composition of the brans and their respective fractions are shown below.

Sample	Dry matter	Protein	Oil	Fibre	Ash	NNE***
CMB*	90.8	15.7	4.1	45.4	5.5	29.3
CMB fractions of the process						
Protein phase	92.9	31.8	7.7	1.1	7.9	51.5
Fibre	92.8	13.6	3.0	76.9	4.1	2.4
SMB**	89.1	14.3	2.3	23.7	3.2	56.5
SMB fractions of the process						
Protein phase	93.9	27.8	1.5	0.9	3.4	66.4
Fibre	94.3	22.5	4.1	64.8	1.6	7.0

* Conventional milling bran
** Short milling bran
*** Non-nitrogen extracts

EXAMPLE 2

Wheat bran produced from conventional milling was subjected to enzymic tretament and wet milling as described in Example 1. The hydrolysed bran was fractionated using a two-phase decanter into an insoluble (combined fibre and aleurone) and a soluble fraction.

The soluble fraction was fed into a separator for fractionation using centrifugal forces thus producing two phases. The germ-rich phase was washed with water and fed again to the separator to remove the excess sugars and other light contaminants. The resulting protein fraction was kept as such or mixed with evaporated liquid whey on a 1:1 ratio (dry matter basis).

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The endosperm-rich fraction, which was heavily contaminated with sugars, was fed to an ultrafilter in order to separate low molecular weight sugars and a protein fraction with less sugar contamination.

All soluble protein fractions, i.e. germ and endosperm-rich phases and the mixtures with whey, were spray dried separately. The sugar fraction was concentrated by vacuum evaporation at mild temperature (t = 60 °C) until a 75% sugar concentration was achieved. The fibre fraction was oven dried.

The additional washing carried out on both germ-rich protein and fibre fraction was very effective to decrease the amount of light soluble contaminants from each fraction, and therefore increase the relative content of valuable components.

Compositional data indicates that germ and endosperm-rich protein fractions have a different relative content of protein and oil. Protein and oil content from the former were 48.6 and 18.6%, respectively and those from the latter were 28.7% and 1.5%, respectively. The insoluble phase containing primarily the bran pericarp (fibre) and the aleurone proteins had 86.4% fibre and 12.6% protein. The chemical composition of the germ-rich phase – whey mix was 31.5% protein, 9.8% oil and 37% lactose.

A further important observation was that the spray dried germ-rich fraction containing 18.6% oil was substantially more resistant to oxidation (rancidification) compared to the original wheat bran. The original wheat bran started getting rancid after 3 weeks of storage. Despite the fact that no exogenous anti-oxidants were added to the germ-rich fraction it only started going off after 12 weeks of storage.

EXAMPLE 3

Previous examples illustrate the use of starch-hydrolysing enzymes and wet milling followed by various separation steps in order to yield both protein, sugar and fibre fractions, the latter still containing substantially high amounts of aleurone proteins. It could be of interest for same applications to separate, at least partly, the aleurone proteins from the bran

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pericarp (fibre) and recover such proteins in the same fraction as the endosperm-rich fraction for instance.

A trial was set up in the same way as described in EXAMPLE 2, except that a cocktail of polysaccharidases containing both high cellulase and xylanase activities was added together with the amyloglucosidases, and let to work for 3 h. Temperature and pH conditions were kept unchanged. The resulting reaction mixture was further treated exactly as described in EXAMPLE 2.

The inclusion of polysaccharidase during the hydrolysis step had a positive effect with regards to aleurone protein extraction and protein recovery as measured by the mass balance and protein content. The protein content in the endosperm-rich fraction increased from 28.7% (without polysaccharidases) to 34.7% (with polysaccharidases) and the overall protein recovery was increased by 35% when polysaccharidase was added.

EXAMPLE 4

The colour of protein ingredients can be of importance particularly in some food and feed applications. Milk products such as caseinates, whey powder and whey protein concentrate have a light colour and soy protein concentrate have a light brown colour. These products are the main ingredients in high value feeds such as calf milk replacer. But, in same food applications such as sausage and hamburger despite the fact the inclusion level is much lower, colour can still play an important role in the product acceptability.

The technical feasibility of bleaching the germ-rich fraction was assessed by two means. 1. Solely alkali and hydrogen peroxide bleaching, and 2. Alkali-free peroxidase and hydrogen peroxide bleaching.

1. Ten g samples of germ-rich fraction were incubated in 1 L beakers containing 100 ml water. Samples were dispersed with stirring and ca. 0.25ml NaOH added until pH 12 was reached. Solutions were warmed at 50 °C and 3.5, 5 and 10 ml of 30% H_2O_2 were added to different flasks to provide uptake levels 10, 15 and 30% H_2O_2 on weight basis of germ-rich fraction. Mixtures were stirred for 1 h and neutralise with acetic acid.

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Full bleaching was achieved with 15 and 30% H_2O_2. Sample treated with 10% H_2O_2 was only partly bleached. All alkali bleached samples became darker with drying.

2. Ten g sample of germ-rich fraction was incubated in 1 L beaker containing 100 ml water. Samples were dispersed with stirring and ca. 0.25 ml NS 51004 Novozymes peroxidase was added. Solution was warmed at 50 °C and 3.5 of 30% H_2O_2 was added to the flask, i.e. 10% H_2O_2 on weight basis of germ-rich fraction, and the mixture stirred for 2 h.

The peroxidase - hydrogen peroxide bleaching was effective, consumed less chemicals and no darkening of the sample was observed after drying.

EXAMPLE 5

Amongst the various end-uses of the germ-rich fraction one could describe meat products such as hamburgers, sausages and meat balls. In such end-uses germ-rich fraction could replace meat, soy protein concentrate and isolate, but also milk casein and caseinates, to mention just a few. It is therefore important to test the overall performance of the germ-rich fraction with regards to emulsifying and binding capacity, taste, etc.

A trial set up to test the feasibility of incorporating various germ-rich fractions extracted from wheat bran into a traditional meat ball recipe consisted of meat, garlic, premix and water.

The following spray dried fractions were tested:

- Germ-rich fraction extracted from short milling wheat bran - (I)
- Germ-rich fraction extracted from conventional milling wheat bran - (II)
- 1:1 mix of whey and II, on dry matter basis - (III)

Meat ball recipes were tested without germ-rich fraction (control recipe) or with 2.5% inclusion of samples I, II or III. Meat balls were analysed for weight loss, taste, texture and colour after frying.

The results are described in the table below.

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Recipe tested	Weight loss after frying (%)	Colour	Texture
Control (meat, garlic, premix and water)	8.8	Reference	Reference
Control + 2.5% of I	6.7	Slightly darker	Slightly tougher
Control + 2.5% of II	6.1	Similar	Similar
Control + 2,5% of III	4.9	Similar	Slightly more tender

The overall conclusion was that the samples performed well as additives in a meat ball recipe, and were particularly interesting as they all decreased the weight loss after frying.

END-USES

Germ-rich fraction

The high protein content of the germ-rich fractions makes it an ideal substitute for existing expensive proteins from animal and vegetable origin. Additionally, the germ-rich fraction because of the nature of its protein, the presence of high quality oil and phospholipids also exhibit interesting functionalities such as emulsification, texture and binding.

One can list, as examples, the following existing products, which can be replaced by the germ-rich fraction in the food industry:

Animal protein: casein and caseinates, plasma protein and egg white
Vegetable proteins: soy protein concentrates and isolates, texturized soy, hydrolysed gluten and potato protein,

Generally, the above products can be used as meat extenders and texturizer ingredients in hamburger, sausage, and meat balls production to mention a few. Or, as a casein replacer in the production of sausage, spreads, dressings, etc.

In the feed industry, the germ-rich fraction is an ideal ingredient for high value feeds such as calf milk replacer, starter feeds for calves, piglets and chicks, fish feeds and pet food. In such applications it can substantially replace the use of soy proteins (texturized soy, concentrate and isolate), potato protein, hydrolysed gluten, high quality fish meal, plasma protein, and dry milk products such whey protein concentrate, whey and skimmed milk.

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Endosperm-rich fraction

The endosperm-rich fraction can be used in the food industry primarily as a gluten replacer, but also to partly substitute soy proteins in the production of various meat and vegetable products. In feed applications, it can partly replace gluten, soy and milk proteins as an ingredient to calf milk replacer, piglet started feed and fish feed.

Fibre fraction

The fibre fraction consisted of pericarp and aleurone can be used as an interesting source of gluten- and phytate-free fibre source to replace conventional cereal brans. The main end-uses as food would be in the baking industry and breakfast cereal.

A preferred embodiment of a plant for carrying out the invention is shown in the attached drawing wherein 1 denotes a suspension and hydrolysis vessel 1 and connected to a wet mill 2 which by its milling action increases the active surface of he hydrolysis. The slurry is allowed to pass the wet mill 2, 1 to 3 times. The slurry is then transferred to a 2-phase decanter 6 via a heat exchanger 3, which decanter 6 separates the bran and liquid (water and protein) phases. The bran having a dry matter content of about 40%, is then further washed once using water in a suspension vessel 4, and is allowed to pass a second decanter 6, again. Then a pure bran obtained has a dry matter content of 95% after drying in a ring drier 10. The collected liquid phases, having a dry matter content of 5%, are transferred to a separator 7, via a mixing tank 5, in which separator 7 insoluble protein is separated off. The liquid phase from the separator 7, having a dry matter content of 2%, and comprising water and soluble proteins is allowed to pass an ultra filter 8 having a molecular cut of 50 kD. Depending on different requirements this cut can vary between 20 and 100 kD. After the ultra filter 8 a liquid phase is obtained which is evaporated in an evaporator 9, and concentrated to a syrup, having the dry matter content of 75%. The concentrate of the ultra filtration is either isolated as such, or added to the fraction of insoluble proteins, which latter has a dry matter content of 9.5%. The protein-sugar fraction obtained is spray-dried in a spray drier 11 to provide a concentrated, dried protein-sugar fraction, and the protein-oil fraction is dried in a spray-drier 11 to provide a concentrated, dried protein-oil fraction.

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CLAIMS

1. Process for the wet fractionation of cereal bran components, characterized in that bran is first subjected to a combination of enzymic treatment with enzymes of the group starch- and phytate-hydrolysing enzymes, and wet milling, followed by an optional step of enzyme inactivation by wet heat treatment, and the next step whereby the insoluble phase containing both pericarp and aleurone fractions are separated by centrifugal forces from the aqueous phase containing germ and residual endosperm components, and that the aqueous phase is further separated by centrifugal force into a germ-rich fraction and an endosperm-rich fraction, and that the proteins contained in the endosperm-rich fraction are concentrated.

2. Process according to claim 1, wherein cereal brans are the fibrous-residue resulting from a primary grain milling, i.e. after the separation of the endosperm fraction, of wheat, rice, barley, oat, rye and triticale, and having variable chemical compositions, presence of anti-nutritive factors, and presence of various anatomical fractions, i.e. pericarp, germ, and residual endosperm.

3. Process according to claim 1, wherein the combination of wet milling with enzymic treatment is arranged to increase substrate accessibility thereby improving the overall hydrolysis performance and the subsequent separation of both insoluble and soluble fractions of varied density/solubility.

4. Process according to claim 1, wherein the enzymic treatment is accomplished by using amylases and/or amyloglucosidases, polysaccharidases (cellulases, beta glucanases, xylanases and pectinases for instance) or by a combination of such enzymes and phytases.

5. Protein fraction derived substantially from the germ and produced according to claims 1-4, wherein the said fraction contains at least 40% protein and 10% oil on dry matter basis and exhibits an increased shelf life with regards to resistance to oxidation compared to the original bran, and that the said fraction contains less than 1% fibre, and it retains the emulsifying capacity of wheat germ.

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6. Protein fraction according to claim 5,
wherein liquid whey is incorporated in to the said fraction at levels varying from 20 to 80% by weight on dry matter basis, and that the final mixture is dried.

7. Protein fraction derived substantially from the residual endosperm and produced according to claims 1-4,
wherein the said fraction contains at least 35% protein and 10% sugar and less than 2% oil and 1% fibre.

8. Protein fraction according to claim 7,
wherein liquid whey is incorporated in to the said fraction at levels varying from 20 to 80% by weight on dry matter basis, and that the final mixture is dried.

9. Fibre fraction produced according to claims 1-4,
wherein the said fraction consists of cell wall components of bran (>85%) and aleurone proteins (>10%), and substantially free of gluten and starch.

10. Sugar fraction produced according to claims 1-4,
wherein the said fraction is originated primarily from the residual endosperm and it contains more than 65% sugars (such as glucose, maltose and malto-triose) on dry matter basis.

11. Use of a protein fraction, as described in claim 5-6, in feed and food applications to replace other protein products from vegetable and animal sources.

12. Use of a protein fraction, as described in claim 7-8, in feed and food applications to replace other protein products from vegetable and animal sources.

13. Use of a fibre fraction, as described in claim 9, in feed and food applications to replace other insoluble fibrous products or as a raw material for further processing.

14. Set up for carrying out the process according to claims 1-4,
chacterized in

that it comprises a hydrolysis vessel (1), a wet mill (2), a heat exchange for enzymic innactivation (3), washing tanks (4), a mixing tank (5), a decanter (6), a separator (7), an ultra-filter (8), an evaporator (9), and optionally dryers (10).

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ABSTRACT

A process for the fractionation of valuable fractions from cereal brans (e.g. wheat, barley and oat brans, and rice polish) is described. In particular, this invention describes a two step process, in which the said bran is first subjected to a combination of enzymic treatment and wet milling, and a second step consisting of sequential centrifugation and ultrafiltration, which aims at physically separating the main bran fractions, i.e. insoluble phase (pericarp and aleurone layer), germ-rich fraction, residual endosperm fraction and soluble sugars. This invention also describes a process that minimises the recovery of unwanted components, e.g. fibre as a contaminant to non-fibrous fractions and phytates, in the resulting fractions, and that such fractions retain the beneficial properties, e.g. high nutritive value, anti-oxidating capacity and emulsifying capacity, which was originally present in the grain.

(FIG)

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Cereal Bran Fractionation Process